US-CL-CURRENT: 424/241.1,424/275.1 ,424/282.1 ,424/810 ,514/885 ,530/868

US-PAT-NO: 5681571
DOCUMENT-IDENTIFIER: US 5681571 A
TITLE: Immunological tolerance-inducing agent
DATE-ISSUED: October 28, 1997
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Czerkinsky; Cecil	Goteborg	N/A	N/A	SEX

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ABSTRACT:
An immunological tolerance-inducing agent comprising a mucosa-binding molecule linked to a specific tolerogen is disclosed. Further, a method of inducing immunological tolerance in an individual against a specific antigen, including hapten, which causes an unwanted immune response in said individual comprising administration by a mucosal route of an immunologically effective amount of an immunological tolerance-inducing agent of the invention to said individual, is described.

27 Claims, 0 Drawing figures
Exemplary Claim Number: 1

BSPR:
The way in which the specific tolerogen is linked to a mucosa-binding molecule in an immunological tolerance-inducing agent of the invention is not important as long as said tolerogen and said molecule can perform their respective function. Thus, they may be linked to each other directly by simple chemical procedures. Chemical procedures to couple proteins such as the B subunit of cholera toxin (CTB) or the thermolabile **enterotoxin** of Escherichia coli (LTB) to lipids, haptens, carbohydrates, nucleic acids as well as to other proteins including antibodies and synthetic peptides are well known in the art (e.g. see Carlsson, J. et al 1978. Biochem. J. 173:723-737; Cumber, J. A. et al. 1985. Methods in Enzymology 112:207-224; Walden, P. et al. 1986. J. Mol. Cell Immunol. 2:191-197; Gordon, R. D. et al. 1987. Proc. Natl. Acad. Sci. (USA) 84:308-312; Avrameas, S. and Ternynck, T. 1969. Immunochemistry 6:53; Joseph, K. C., Kim, S. U., Stieber, A., Gonatas, N. K. 1978. Proc. Natl. Acad. Sci. USA 75:2815-2819; Middlebrook, J. L. and Kohn, L. D. (eds): 1981. Receptor-mediated binding and internalization of toxins and hormones. Academic Press, New York, pp 311-350). The tolerogen can also be fused genetically to the CTB (or LTB) gene (Sanchez, J., Svennerholm, A-M and Holmgren, J. 1988. Genetic fusion of a non-toxic heat-stable **enterotoxin**-related deca-peptide antigen to cholera toxin B subunit. FEBS Letters 241:110-114) and the resulting chimeric gene then be expressed in a suitable expression system, such as a bacteria, a yeast or a virus. Alternatively, the tolerance inducing agent may comprise a fragment of a nucleic acid sequence (DNA or RNA) or a synthetic polynucleotide encoding the tolerogen which is then chemically coupled to the mucosa-binding molecule and administered by the mucosal route, advantage being then taken of the capacity of cells from host mucosal tissues to ensure transcription and/or translation of the corresponding gene into a mature protein (Rohrbaugh, M. L. and McGowan, J. J. 1993. Gene-transfer for therapy

-1 infection. Ann. N.Y. Acad. Sci. Vol 685, pp
and Felgner, P. L., 1993. Direct gene-transfer for
munization. Trends in Biotechnology Vol 11 No. 5, pp
L. Hunt, L. A., Webster, R. G. 1993. Protection against
challenge by immunization with a
ng plasmid DNA. **Vaccine** 11:957-960; Martinon, F., Krishnan, S., Lenzen, G., Magne, R., Gomard, E., Guillet, J. G., Levy, J. P. and Meulien, P. 1993. Eur. J. Immunol. 23:1719-1722). Yet other alternative presentation forms could consist in the incorporation of the tolerogen or its nucleic acid precursor into a protective vehicle such as a liposome or equivalent biodegradable vesicles onto which the mucosa-binding substance had been or shall be attached allowing efficient binding of the tolerogen-containing vehicle to a mucosal surface for improved tolerogenic efficacy. With this type of presentation form, the tolerogen may be either free or linked to another molecule.

BSPR:
Recombinant cholera toxin B subunit (CTB) was produced in a mutant strain of Vibrio cholerae deleted of the cholera toxin genes and transfected with a plasmid encoding the CTB subunit (Sanchez, J. and Holmgren, J. 1989. Recombinant system for over-expression of cholera toxin B subunit in Vibrio cholerae as a basis for **vaccine** development. Proc. Natl. Acad. Sci. USA 86:481-485). Recombinant B subunit of Escherichia coli heat-labile **enterotoxin** (LTB) was produced similarly in a mutant strain of Vibrio cholerae deleted of the cholera toxin genes and transfected with a plasmid encoding E. coli LTB (Hirst, T. R., Sanchez, J. Kaper, J. B., Hardy, S. J. S, and Holmgren, J. 1984. Mechanism of toxin secretion by Vibrio cholerae investigated in strains harbouring plasmids that encode heat-labile **enterotoxins** of Escherichia coli. Proc. Natl. Acad. Sci. USA 81:7752-7756). In these expression Systems, CTB and LTB are recovered from bacterial growth media as secreted proteins. Bacterial cultures were centrifuged at 8000 rev per min for 20 min and the supernatants were collected and adjusted to pH 4.5 with dilute HCl. After precipitation with hexametaphosphate (final concentration 2.5 g/l) for 2 hours at 23.degree. C. followed by centrifugation at 8000 rev per min, the pellets were dissolved with 0.1M sodium phosphate buffer, pH 8.0 and dialysed against 0.01M phosphate-buffered saline, pH 7.2, The dialysate was then centrifuged at 15 000 rev per min to remove residual insoluble material and the supernatant was further clarified by filtration through a 0.22 .mu.m filter (Millipore, Bedford, Mass.). Finally, CTB and LTB were purified by standard gel filtration chromatography through columns of Sephadex G-100 (Pharmacia, Sweden).

CLPR:
1. A method of inducing immunological tolerance in a mammal to a T-cell-associated immunological response, which comprises administering by a mucosal route to a mammal suffering from or prone to a T-cell associated disease an immunological tolerance-inducing agent, wherein said agent comprises (i) a mucosa-binding molecule selected from the group consisting of the B subunit of cholera toxin and the B subunit of **heat-labile** enterotoxin of Escherichia coli, linked to (ii) a specific tolerogen associated with said T-cell associated immune response, and wherein said agent is administered in an amount and for a time effective to induce tolerance against said T-cell associated immune response.

CLPR:

CT & LTII

(FILE 'HOME' ENTERED AT 16:56:59 ON 26 FEB 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 16:57:18 ON 26 FEB 2002

L1	5	S ENTEROTOXINS AND DNA VACCINE
L2	3	DUP REM L1 (2 DUPLICATES REMOVED)
L3	885	S ENTEROTOXINS AND (IMMUNIZATION OR VACCINE)
L4	0	S L3 AND (CHLOERA TOXIN B/A2)
L5	0	S L3 AND (CHLOERA TOXIN)
L6	0	S CHLOERA TOXIN B
L7	226	S CHOLERA TOXIN AND L3
L8	45	S L7 AND SUBUNITS
L9	27	DUP REM L8 (18 DUPLICATES REMOVED)
L10	12	S L9 AND 1995-2000/PY
L11	6	S HEAT-LABILE TOXIN II
L12	1	S L3 AND L11
L13	3772	S RUSSELL M?/AU OR CONNELL T?/AU
L14	13	S L3 AND L13
L15	4	DUP REM L14 (9 DUPLICATES REMOVED)

L Number	Hits	Search Text	DB	Time stamp
7	3	"5800821"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:07
1	2	"9958145"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:17
13	237	enterotoxin same vaccine	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:18
19	29	heat$labile with II	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:19
31	7	((enterotoxin same vaccine) and (heat$labile with II)) and subunit$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:19
37	318	cholera adj toxin adj B	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:21
43	0	(cholera adj toxin adj B) same subunit$ same A2/B	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:21
49	245	(cholera adj toxin adj B) same subunit$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:22
67	2	(cholera adj toxin adj B) same subunit$ same adjuvant same expression	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:22
61	1	(cholera adj toxin adj B) same subunit$ same adjuvant same plasmid	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:24
25	8	(enterotoxin same vaccine) and (heat$labile with II)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:25
55	62	(cholera adj toxin adj B) same subunit$ same adjuvant	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:32

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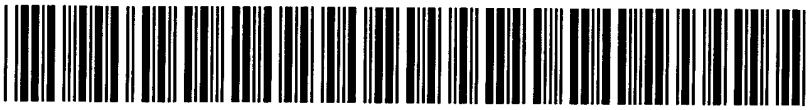

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